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14. ABSTRACT

The purpose of this study was to identify the genetic polymorphisms in nicotinic acetylcholine (ACh) receptors (nAChRs) that modify the risk for non-small cell lung carcinoma (NSCLC). The proposal addressed two areas of emphasis: 1) understanding the molecular mechanisms that lead to clinically significant lung cancer; and 2) identification of the mechanisms that lead to the development of the various types of lung cancer. This application was based upon our recent discoveries of both positive and negative associations of single nucleotide polymorphisms (SNPs) of certain nAChR subunits with NSCLC, and the ability to mutant receptors to increase or decrease susceptibility of bronchial cells to the tobacco nitrosamine-induced carcinogenic transformation of human bronchial cells [1-2].

15. SUBJECT TERMS
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Introduction

The purpose of this study was to identify the genetic polymorphisms in nicotinic acetylcholine (ACh) receptors (nAChRs) that modify the risk for non-small cell lung carcinoma (NSCLC). The proposal addressed two areas of emphasis: 1) understanding the molecular mechanisms that lead to clinically significant lung cancer; and 2) identification of the mechanisms that lead to the development of the various types of lung cancer. This application was based upon our recent discoveries of both positive and negative associations of single nucleotide polymorphisms (SNPs) of certain nAChR subunits with NSCLC, and the ability to mutant receptors to increase or decrease susceptibility of bronchial cells to the tobacco nitrosamine-induced carcinogenic transformation of human bronchial cells [1-2].

Body

According to the Statement of Works, we performed next generation sequencing of DNA samples obtained from the Ontario Tumor Bank at the UC Irvine's Genomics High Throughput Facility. The obtained results will be analyzed at the Bioinformatics Core Facility closely linked with the Genomics High-Throughput Facility and the Institute for Genomics and Bioinformatics. Evaluation of lung tissue levels of the nAChR variants associated with increased or decreased susceptibility to lung cancer at the mRNA and protein level was not performed because isolation of DNA consumed most of the tissue sample, whereas purchasing of additional samples was not a part of the limited budget of this pilot project. Targeted resequencing of the human nAChR subunits α1, α2, α3, α4, α5, α6, α7, α9, α10, β1, β2, β3, β4, δ and ε was performed in lung samples from cancer patients. Approximately 410kb of sequence consisting of 175 exons was targeted using the Agilent SureSelect technology. Libraries were multiplexed together and sequenced using the Illumina HiSeq 2500 platform technology. The resulting reads were analyzed for quality control and aligned to human hg19 targeted regions for the nAChR genes under consideration. Variants were identified for each sample by comparison to the reference genome using a quality based approach. The SNPs were annotated according to exon/intron, the amino acid change and by database SNP "rs" numbers. The variants from the test samples were combined and annotated with frequency occurrence. Over 4800 total variants were identified from the thirty-five cancer samples from 410kb of targeted sequence at a frequency of at least 20%. The majority of the variants were within the coding regions of the genes and approximately 6% were within introns. Annotation to the SNP database identified 434 of these variants as having been previously annotated. There were fifteen variants that resulted in an amino acid change when compared to the human reference genome. Of the fifteen, nine were associated with CHRNB4 nicotinic receptor and five of the nine variants were present in all thirty-five samples analyzed (*see* **Table**). These five variants in CHRNB4 are within ~100 bases of each other and four have not previously been annotated into the SNP database.

Key Research Accomplishments

In this pilot study, we identified novel lung cancer-specific mutation in the β4 nAChR subunit that may play an important role in an increased lung cancer susceptibility among the carriers of this mutation.

Reportable Outcomes

The data obtained in this pilot study will become reportable upon completion of the studies on a larger number of lung cancer and control samples mandated by the epidemiological analysis.

The results obtained in this proposal provided preliminary data for an R01-type NIH grant application for the January 5, 2014 submission deadline.

Conclusion

The SNPs of β4 nAChR subunit found n 100% of lung cancer samples may be responsible for aberrant expression of the nAChR subtypes associated with NSCLC. Elucidation of genetic predisposition to lung cancer based on this new nAChR polymorphism improves our understanding of the mechanisms involved in early stages of lung carcinogenesis, and lay a groundwork for identification of susceptible individuals and development of personalized approaches to lung cancer prevention and treatment.

References

1. Chikova A, Grando SA: Naturally occurring variants of human α9 nicotinic receptor differentially affect bronchial cell proliferation and transformation. PLoS ONE 2011;6 (11) e27978.

2. Chikova A, Bernard H-U, Shchepotin IB, Grando SA: New associations of the genetic polymorphisms in nicotinic receptor genes with the risk of lung cancer. Life Sci 2012; 91: 1103-1108.

Appendices

The Table of nAChR subunit SNPs found to be most prevalent in tested lung cancer samples is attached.

	Gene	Chromosome	Region	Type	Reference	Allele	dbsnp variants*	Sample count	Sample frequency	Amino acid change
1	CHRNB4	15	78927870	SNV	G	A	rs72648898	35	100.0	ENSP00000416386:p.[Arg39Cys]; ENSP00000261751:p.[Arg39Cys]; ENSP00000457404:p.[Arg36Cys]
2	CHRNB4	15	78927927	SNV	T	C		35	100.0	ENSP00000416386:p.[Asn20Asp]; ENSP00000261751:p.[Asn20Asp]; ENSP00000457404:p.[Asn17Asp]
3	CHRNB4	15	78927912	SNV	T	G		35	100.0	ENSP00000416386:p.[Asn25His]; ENSP00000261751:p.[Asn25His]; ENSP00000457404:p.[Asn22His]
4	CHRNB4	15	78927810	SNV	G	C		35	100.0	ENSP00000416386:p.[Gln59Glu]; ENSP00000261751:p.[Gln59Glu]; ENSP00000457404:p.[Gln56Glu]
5	CHRNB4	15	78927918	SNV	C	T		35	100.0	ENSP00000416386:p.[Val23Met]; ENSP00000261751:p.[Val23Met]; ENSP00000457404:p.[Val20Met]
6	CHRNB4	15	78927816	SNV	T	C		33	94.3	ENSP00000416386:p.[Lys57Glu]; ENSP00000261751:p.[Lys57Glu]; ENSP00000457404:p.[Lys54Glu]
7	CHRNA9	4	40356422	SNV	A	G	rs10009228	32	91.4	ENSP00000312663:p.Asn442Ser
8	CHRNB4	15	78913068..78913070	Deletion	CAG	-	rs66793222	28	80.0	ENSP00000267951:p.[Leu23del]; ENSP00000315602:p.[Leu23del]; ENSP00000452896:p.[Leu23del]
9	CHRNA6	8	27324822	SNV	T	C	rs891398	28	80.0	ENSP00000385026:p.[Thr125Ala]; ENSP00000429616:p.[Thr125Ala]; ENSP00000240132:p.[Thr110Ala]; ENSP00000430612:p.[Thr125Ala]
10	CHRNB4	15	78882925	SNV	G	A	rs16969968	24	68.6	ENSP00000299565:p.Asp398Asn

11	CHRNB4	15	30665281..30665282	Deletion	CA	-	rs67158670, rs201490160	18	51.4	ENSP00000299847:p.[Leu76fs]; ENSP00000455401:p.[Leu76fs]
12	CHRNB1	17	7348625	SNV	A	G	rs17856697	17	48.6	ENSP00000461402:p.[Glu32Gly]; ENSP00000304290:p.[Glu32Gly]
13	CHRNA6	8	27328511	SNV	G	A	rs2472553	11	31.4	ENSP00000385026:p.[Thr22Ile]; ENSP00000429616:p.[Thr22Ile]; ENSP00000240132:p.[Thr22Ile]; ENSP00000430612:p.[Thr22Ile]; ENSP00000430422:p.[Thr22Ile]; ENSP00000430856:p.[Thr22Ile]; ENSP00000429953:p.[Thr22Ile]
14	CHRNA9	4	40356041	SNV	C	T	rs55633891	8	22.9	ENSP00000312663:p.Ala315Val

* homo sapian (hg19)